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Occurrence of *Venturia inaequalis* races in Poland able to overcome specific apple scab resistance genes

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Abstract The aim of this study was to confirm the presence of races in populations of the fungus *Venturia inaequalis* that are able to overcome specific apple scab resistance gene(s) within the major apple-growing areas of Poland. The monitoring was conducted in six orchards located in the north, centre and south of Poland. The study involved the use of 16 differential genotypes for pathogenicity testing conducted under both greenhouse and orchard conditions. In addition, the occurrence of apple scab on 10 apple cultivars containing the *Rvi6* gene was assessed in four organic orchards in central Poland. Apple scab was found on their leaves for the first time in Poland in 2010. The use of differential genotypes containing specific resistance genes suggested that 10 apple scab resistance genes had been overcome by *V. inaequalis* in the orchards monitored in this study.

Keywords *Malus* · Avirulence gene · *Venturia inaequalis* · Physiological races

Introduction

Apple scab (*Venturia inaequalis* [Cooke] Wint.) is one of the most serious diseases of apple trees in many apple-growing regions. It causes damage to the leaves and fruits, with negative consequences for fruit quality and yield. Currently, the most common way to protect apple trees against this disease is the application of fungicides (MacHardy 1996; Rossi et al. 2007; Holb 2009). In Poland, during years which have had particularly favourable conditions for the development of apple scab, up to 20 fungicide treatments were found to be necessary in order to avoid losses (Meszka and Masny 2006; Masny 2015). It is possible to reduce the use of fungicides in orchards by cultivating less susceptible or resistant apple cultivars.

Studies investigating the genetic background of resistance and the breeding of apple trees for resistance were first undertaken in the USA. At the beginning of the last century, an American fruit grower, Charles S. Crandall, gathered a large collection of species and clones of wild apple trees and began an intensive study on crossbreeding them with cultivated apple cultivars (Crandall 1926). He observed that clone 821 of the wild species *Malus floribunda* was completely resistant to apple scab. In the USA in the 1940s, two seedlings derived from this clone were used in the PRI breeding-for-resistance programme as part of a joint project between the Purdue, Rutgers and Illinois universities (Janick 2006). This work led to the selection of the 'Prima' cultivar. This cultivar was the first bred by the PRI programme with a satisfactory fruit size and quality (Dayton and Mowry 1970). In the second half of the twentieth century, other countries had also begun to breed for apple scab resistance (Kozlovskaya et al. 2000, Sansavini et al. 2002).

However, studies have shown that the degree of resistance of some newly bred genotypes has changed

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over time, resulting in the adaptation of *V. inaequalis* and breaking down of existing resistance (Williams and Brown 1968; Roberts and Crute 1994; Bénaouf and Parisi 2000). Some of the first studies to investigate physiological races confirmed that isolates of *V. inaequalis* found in different regions of Canada had different virulences towards a particular apple cultivar or group of cultivars (Julien and Spangelo 1957). Other studies conducted within the same period in the USA (Shay and Williams 1956; Shay et al. 1962) demonstrated that many clones derived from different species of *Malus* collected from far-apart regions of the world (Australia, England, Yugoslavia, Germany, Norway and the USA), which were all infected with isolates of *V. inaequalis*, but showed different susceptibilities to the disease. They allowed for the identification and characterisation of four physiological races of *V. inaequalis* which infected specific apple genotypes and formed conidia. According to Shay and Williams (1956) race (1) was commonly observed in the USA and many other countries, race (2) was identified in South Dakota, and race (3) was found in Nova Scotia. Race (4) of *V. inaequalis* was detected at Purdue University in the USA on scab-resistant seedlings of R12740-7 A belonging to *M. pumila* (Williams and Kuć 1969; MacHardy 1996). Studies by Williams and Brown (1968) contributed to the detection and characterisation of race (5) of *V. inaequalis*, which was capable of infecting apple cultivars derived from *Malus micromalus* and *Malus atrosanguinea* which carried the *Rvi5* (*Vm*) resistance gene. Race (6) of *V. inaequalis* was first described by Parisi et al. (1993) and is distinguished from the other five races of *V. inaequalis* by its capacity to cause sporulating spots of scab on certain apple hybrids carrying the *Rvi6* gene. The pathogenicity of this race was investigated by Parisi and Lespinasse (1996) on various apple clones. In the 1990s, race (7) of *V. inaequalis* was detected in England and described by Roberts and Crute (1994). An isolate from a naturally infected tree of *M. floribunda* also produced symptoms on *M. floribunda* 821 and other cultivars with the *Rvi6* gene, while other cultivars with the same gene were not infected. The discovery of race (8) of *V. inaequalis* was the result of wider research into the interaction between *V. inaequalis* and apple trees, and was derived from a population of *V. inaequalis* in New Zealand (Bus et al. 2005). The first reports the interactions in the pathogen-plant system concerned rust and flax, and were related to the theory of the complementarity of genes (the gene-for-gene theory) proposed by Flor in the 1940s (Flor 1971). According to this concept, each resistance gene (*R*) of the host plant has a counterpart in the genome of the pathogen, which determines its avirulence. In the case of apple, the presence of the *Rvi* gene, which encodes resistance to scab, makes it possible to recognise an attack by the fungus *V. inaequalis* due to the presence of the specific avirulence gene (*AvrRvi*), making it unable to infect this apple genotype. The presence of resistant apple genotypes in a given ecosystem (environment) exerts selection pressures on the population of *V. inaequalis*, and the resulting adaptations (e.g. mutations) of the pathogen helps to avoid its recognition when infects an apple tree, leading to the emergence of new physiological races.

The definition of a physiological race, as proposed by Gessler et al. (2006), refers to isolates capable of infecting and producing spores on a specific host. After detecting and describing race (6), Parisi et al. (1993) added host h(6), which carries the *Rvi6* (*Vf*) gene, to the five existing differential hosts. This resulted in the identification of the six physiological races of *V. inaequalis*. The set of differential hosts has since been extended to include one for each of the scab resistance genes described in the new nomenclature system (Bus et al. 2009). Over the following years, the set of differentiated hosts was modified and broadened by Bus et al. (2011). The host h(11), represented by *M. baccata jackii*, was replaced by the genotype A722–7 (*M. baccata jackii* × 'Starking'), and four hosts were added to the set: h(14) (Dülmener Rosenapfel), h(15) (GMAL2473), h(16) (MIS op 93.051 G07–098) and h(17) ('Antonovka', APF22). This new system better reflected the emerging complexities associated with the different combinations of genes, both in the host and the pathogen. Recently, the set of differential *Malus* hosts was complemented by a set of *V. inaequalis* reference isolates, characterised by their virulence (Caffier et al. 2015). Patocchi et al. (2009) started the VINQUEST initiative (www.vinquest.ch) to monitor apple scab strains able to overcome a set of apple scab resistance genes, which are currently used, or could be used, in apple breeding.

The aim of this study was to assess the virulence diversity of *V. inaequalis* populations in selected orchards and the identification of physiological races in the major fruit-growing areas of Poland. The differential hosts used in our monitoring were based

on the set of *Malus* hosts recommended by the VINQUEST initiative.

Materials and methods

Leaf scab assessment in apple orchards

The study was conducted in four organic apple orchards located 10–60 km apart. The cultivars grown in these orchards included those susceptible to apple scab and those that contain the *Rvi6* gene (Table 1).

In 2010–2012, during the secondary infection period (after completion of *V. inaequalis* ascospore discharge), apple scab symptoms were assessed in all orchards by examining the leaves, leaf petioles, pedicels and fruitlets on 16 randomly selected trees for each cultivar (four replicates for each cultivar). For the determination of the number of leaves with conidia sporulating lesions, detailed observations were performed on 1600 leaves (400 randomly selected leaves in each of the four replicates) originating from the *Rvi6* cultivars only. In cases where such determination was difficult or impossible, the examination was performed in the laboratory using a microscope. The data were statistically analysed by ANOVA after being transformed using the Bliss function, and the results are expressed as a percentage. The differences between means were estimated using the Newman-Keuls test with a 5 % level of significance ($p < 0.05$).

Inoculation of differential hosts in the greenhouse with orchard-derived samples

In 2011–2012, combined inocula consisting of conidia originating from different cultivars, all carrying the *Rvi6* gene, were sampled from three organic orchards, and unprotected apple trees growing in orchards at Dąbrowice and used for the inoculation. Samples of 200 leaves with scab were collected from trees of

Table 1 List of the organic apple orchards located in central Poland which were monitored in this study

Item no.	Symbol	Location	Cultivars		Age of trees (years) at the beginning of the study
			With *Vf* (*Rvi6*)	Without *Vf* (*Rvi6*)	
1.	A	Pęczniew near Sieradz (51°49′19″N, 18°43′23″E)	Ariwa		8
			Biogolden		8
			Goldstar		8
			Rajka		8
			Topaz		8
				Antonovka	8
2.	B	Nowe Rowiska near Skierniewice (51°53′46″N, 20°07′05″E)	Topaz		8
				Papierówka	> 40
				Kronselka	> 40
				Grochówka	> 40
				Malinowa	> 40
				Wealthy	> 40
				Szara Reneta	> 40
3.	C	Nowy Dwór near Skierniewice (51°52′13″N, 20°14′49″E)	Enterprise		8
			Free Redstar		8
			Rubinola		8
			Topaz		6
				Pinova	6
4.	D	Wola Branicka near Zgierz (51°56′46″N, 19°27′32″E)	Freedom		18
			Novamac		18
			Topaz		4

each apple cultivar and placed in separate plastic bags. Immediately after transporting the samples to the laboratory, the conidia of *V. inaequalis* were isolated from the lesions on leaves for each sample by transferring them into a beaker containing 50 ml of distilled water using a small paint brush. The resulting suspensions were filtered through a gauze to remove impurities (e.g. small fragments of leaves). The filtered suspensions were then poured into 15-ml plastic test tubes and frozen at −20 °C until use.

The identification of each of the physiological races of *V. inaequalis* in each population of the monitored orchards was performed during 2011–2012 on the basis of pathogenicity tests on potted trees (grafted onto M.26 rootstocks) of the differential hosts (Table 3), as recommended by the VINQUEST initiative (Patocchi et al. 2009). Prior to inoculation, the youngest fully unfolded leaf on the developing long shoots of each of the differential plantlets were marked to enable accurate assessment of the severity of the disease after the incubation period. Suspensions of conidia were prepared after thawing, and the concentration of conidia was determined using a Bürker chamber. The concentration of the mixed suspensions taken from the different apple cultivars from each location was established to be 10^3 spores per 1 ml of suspension (10^3 spores/ml is a low concentration, but does not appear to have affected the experiments). For each location, one suspension was prepared by combining equal volumes of the suspensions obtained from each of the apple cultivars being monitored in locations A, B and D, and also from the unprotected apple tree cvs. 'Jonagold' and 'McIntosh' which were growing in the orchards at Dąbrowice (location E). Four trees (replicates) of each differential genotype were inoculated by applying the mixed suspensions of conidia with a sprayer connected to a Millipore-type laboratory compressor. Afterwards, the trees were placed for 48 h in a plastic tunnel with a relative humidity (RH) of 95–100 % and a temperature of 17–24 °C, and were maintained under natural lighting. The differential genotypes were then transferred to the greenhouse (RH of at least 80 %, temperature of between 15 and 27 °C with ventilation).

The assessment of infection of the leaves was carried out about 4 weeks after inoculation. After the appearance of sporulating apple scab lesions, the leaves with symptoms of scab and the resistant leaves without any symptoms were counted.

Trapping apple scab on differential hosts under natural conditions

In 2013–2014, assessments were conducted in three commercial orchards located in some of the main apple production regions in Poland, which were managed under the Polish integrated fruit production (also known as Integrated Pest Management - IPM) guidelines (http://piorin.gov.pl/publikacje/metodyki-ip/, http://www.minrol.gov.pl/Informacje-branzowe/Produkcja-roslinna/Ochrona-roslin/Integrowana-ochrona-roslin/Metodyki-integrowanej-ochrony-roslin). These included Brzezna (near Nowy Sącz, Beskid mountains, southern Poland), Dąbrowice (near Skierniewice, central Poland) and Miłobądz (near Gdańsk, northern Poland, close to the Baltic Sea). The orchards consisted predominantly of susceptible apple cultivars, e.g. 'Gala', 'Jonagold' and 'Idared', while the scab-resistant cultivars, such as 'Topaz', were grown in a small area (Table 2). The monitoring of pathogen races was performed using potted trees of the differential hosts grafted onto M. 26 rootstocks (Table 3). Each year, before the release of *V. inaequalis* ascospores, the potted trees of the differential genotypes were placed in designated locations in each orchard. They were not protected with any fungicides. During the period of secondary infection, after the release of ascospores had ended, an assessment of lesions with sporulating mycelium of *V. inaequalis* was carried out on all of the fully developed leaves of these trees.

Scab warning

Critical apple scab periods were determined by the number of hours of leaf wetness at various temperatures, according to the criteria detailed by Mills (1944) as modified by MacHardy and Gadoury (1989), and these periods were recorded with AVI-MET (Polish scab signalling system, TEXA, Kutno) based on the weather data. Weather monitoring was conducted over three consecutive years, from 2010 to 2012, using four meteorological stations located near the monitored orchards. The temperature, precipitation, RH, and leaf wetness were recorded continuously every hour from the beginning of April until the end of September using an automatic weather station (Metos, Pessl Instruments, Weiz, Austria). Meteorological information was obtained from the Soska Konsulting and from the Research Institute of Horticulture.

Table 2 Proportion of apple cultivars in the orchards as guided by the IPM system (expressed as %)

Cultivars	Location		
	Dąbrowice (51°54′48″N, 20°06′24″E)	Brzezna (49°35′55″N, 20°37′08″E)	Miłobądz (54°08′48″N, 18°42′44″E)
Alwa			8
Cortland	2.5		2
Elstar	2		
Enterprise		0.1	
Gala	10	21	13
Gloster	1	9	5
Golden	1	30	3
Delicious		0.1	
GoldStar		3	
Idared	2	8	
Jonagold	53	5.5	7
Ligol	8	6	12
Ligolina	4.5		
Malinowa			1
McIntosh	3		
Prima			0.5
Rajka		0.1	
Rubin		11	
Rubinola	6	0.1	
Spartan			9
Starkrimson			2
Šampion	6.5	6	37
Topaz	0.5	0.1	0.5

Table 3 Differential apple genotypes used in this study

Differential hosts	Name/symbol of apple genotype	Name of resistance gene	
		Former	Current
h(1)	'Golden Delicious'	*Vg*	*Rvi1*
h(2)	TSR34T15	*Vh2*	*Rvi2*
h(3)	F_1 of 'Geneva'[a]	*Vh3*	*Rvi3*
h(4)	TSR33T239	*Vh4*	*Rvi4*
h(5)	9-AR2T196	*Vm*	*Rvi5*
h(6)	'Priscilla'	*Vf*	*Rvi6*
h(7)	*floribunda* 821	*Vf*, *Vfh*	*Rvi7*
h(8)	B45	*Vh8*	*Rvi8*
h(9)	J34 (F_1 of 'Dolgo')	*Vdg*	*Rvi9*
h(10)	A723–6	*Va*	*Rvi10*
h(11)	*Malus baccata jakii*	*Vbj*	*Rvi11*
h(12)	Hansen's baccata #2	*Vb*	*Rvi12*
h(13)	'Durello di Forlì'	*Vd*	*Rvi13*
h(14)	'Dülmener Rosen'	*Vdr1*	*Rvi14*
h(15)	GMAL2473	*Vr2*	*Rvi15*

[a] According to Patocchi personal communication and Vinquest project this genotype assigned as Q71 (http://www.vinquest.ch/monitoring/establishing_network.htm)

Results and discussion

Monitoring of *avrRvi6* race of *V. inaequalis*

The monitoring carried out in four organic orchards in the areas of Sieradz (A), Skierniewice (B and C) and Zgierz (D), detected apple scab on the leaves of apple trees of all the *Rvi6* cultivars included in the study (Table 4).

In 2010, the *Rvi6* cultivars in orchard A exhibited numerous apple scab lesions on leaves as a result of 28 days of favourable conditions for infection (Table 5). Almost 100 % of the leaves on the 'Ariwa', 'BioGolden' and 'Topaz' cultivars were infected with scab, while the 'Goldstar' and 'Rajka' cultivars were less infected, but still at a very high level (about 85 %). In the early stages of fruitlets growth (71 and 72 BBCH scale, according to a system for uniform coding of phenologically growth stages of plants), the symptoms of apple scab were observed both on the surface and petioles, resulting in all of the total fruitlets to fall. At the end of June and throughout July only single fruits that had not been infected during the flowering remained on the monitored apple trees. In orchard A, during the first year of observation (2010), the cultivars were not protected against scab. The following year, from the beginning of the growing season until flowering, 2–3 copper fungicide treatments had been applied. Despite very dry weather conditions that year, much lower severity of the disease was observed on the leaves of each of the cultivars, amounting to approximately 2 %. According to scab warning in this orchard, only 14 days with infection periods were registered (Table 5). In the last season of the observations (2012) performed in orchard A, despite slightly fewer critical apple scab infection periods (Table 5) and two treatments with copper fungicides (20 and 28 April 2012), an increase in the severity of the disease was observed. At the end of the *V. inaequalis* ascospore discharge, the cultivars which had the highest scab severity were 'Ariwa'

Table 4 Occurrence of apple scab on the leaves of scab-resistant apple cultivars carrying the *Rvi6* gene

Apple cultivars at each of the locations	Percentage of infected leaves		
	2010	2011	2012
Pęczniew, near Sieradz (A)			
Ariwa	94.0 b*	2.4 a	31.7 c
Biogolden	97.6 c	2.6 a	27.3 c
Goldstar	87.4 a	1.8 a	8.1 b
Rajka	85.8 a	1.7 a	6.8 a
Topaz	99.5 d	2.7 a	8.8 b
Nowe Rowiska, near Skierniewice (B)			
Topaz	1.3	0.1	0.5
Nowy Dwór, near Skierniewice (C)			
Enterprise	0.1 a	0.7 b	1.8 ab
Free Redstar	0.1 a	0.1 ab	0.8 a
Rubinola	0.1 a	0.02 a	0.5 a
Topaz	0.1 a	0.5 ab	3.6 b
Wola Branicka, near Zgierz (D)			
Freedom	78.2 b	53.5 b	74.0 b
Novamac	93.4 c	69.0 c	99.2 c
Topaz	7.2 a	5.2 a	12.4 a

*Different letters show significant differences between percentage of infected leaves within one orchard and year ($p < 0.05$)

Table 5 Risk of apple scab infection as recorded from four weather stations located near the monitored orchards

Location Year	Number of critical apple scab infections during each period		
	Primary infections	Secondary infections	The whole season
Pęczniew (A)			
2010	23 (28)[a]	28 (29)	51 (57)
2011	11 (14)	26 (29)	37 (43)
2012	18 (19)	33 (34)	5 (53)
Nowe Rowiska (B)			
2010	17 (23)	15 (18)	32 (41)
2011	10 (13)	21 (39)	31 (52)
2012	8 (15)	21 (24)	32 (41)
Nowy Dwór (C)			
2010	9 (13)	19 (24)	28 (37)
2011	7 (7)	28 (40)	35 (47)
2012	13 (16)	16 (21)	29 (37)
Wola Branicka (D)			
2010	18 (24)	26 (29)	44 (53)
2011	12 (12)	26 (33)	38 (45)
2012	15 (20)	19 (22)	34 (44)

[a] The number of days with weather conditions favourable to apple scab critical periods is given in brackets

and ‘BioGolden’ (about 30 %), while a lower disease severity was observed on ‘Goldstar’ and ‘Topaz’ (about 8 %) and the lowest - on ‘Rajka’ (6.8 %). During the advanced ripening of fruits, approximately 2 weeks before harvest, the number of fruits with scab represented only a small percentage (from 0 % to 2.3 %, data not shown) despite a considerable number of scab lesions found on the leaves. This may be related to a lower number of critical periods when compared to the 2010 season (Table 5) in addition to the copper fungicide applications.

In 2010, scab was observed in orchard B on 1.3 % of the leaves of the ‘Topaz’ cultivar, while only 0.1 % and 7.2 % of the leaves were infected on the same cultivar in orchards C and D, respectively. In the following years, the severity of the disease on the leaves of this cultivar in orchard B decreased and did not exceed 0.5 %.

In the first year of the study (2010), apple scab occurred sporadically in orchard C (0.1 % of leaves infected) on all the apple cultivars (‘Enterprise’, ‘Free Redstar’, ‘Rubinola’ and ‘Topaz’). In 2011, infection of the leaves of the apple cultivars in orchard C was more varied (Table 4). The cultivar which showed the least symptoms was ‘Rubinola’, while the cultivar with the most symptoms was ‘Enterprise’. In the last year of the study, the cultivar with the least amount of scab lesions was again ‘Rubinola’, and the cultivar showing the most symptoms was ‘Topaz’.

In 2010, due to the higher number of critical periods (Table 5), the apple scab severity in orchard D was much higher, than in the other orchards in the Skierniewice area. Symptoms of the disease were detected on all three apple cultivars carrying the *Rvi6* resistance gene. The lowest mean number of leaves with apple scab lesions was observed on young ‘Topaz’ trees (7.2 %), and the highest on older ‘Freedom’ (nearly 80 %) and ‘Novamac’ (over 90 %) cultivars. Prior to harvest in orchard D, only a small portion of the fruit on ‘Topaz’ trees were infected by scab (data not shown), in which copper fungicides were applied. During the following season (2011), the apple scab severity in orchard D was the highest of all the orchards monitored, however, it was still slightly lower than the previous year (Table 4).

In contrast, the level of infection on the leaves of 'Topaz' was lower than on the other two cultivars, amounting to approximately 5 %. In 2012, an increase in the severity of apple scab was observed for all the apple cultivars in orchard D when compared to the previous season. The highest frequency of scabbed leaves was recorded for the cultivars 'Freedom' and 'Novamac' (74 % and 92.2 %, respectively), and the lowest - for 'Topaz' (12.4 %). In other countries, apple scab has also been observed on the leaves of apple cultivars carrying the *Rvi6* gene (Parisi et al. 1993; Vavra and Bocek 2010).

The 2010 monitoring of apple scab in selected apple orchards showed, for the first time, that the apple scab resistance gene *Rvi6* had been overcome in these specific regions of Poland. This discovery coincided with the occurrence of very favourable weather conditions for the disease development. In central Poland, from 13 to 28 days were recorded for apple scab infection periods during the primary season. Summarizing, the changing level of apple scab severity during 3 years of study was related to the number of critical periods in particular years and orchards.

In populations of *V. inaequalis* in five monitored orchards (three organic and two IPM), the genotypes able to infect the apple leaves of cv. Priscilla/h(6) and cause single sporulating lesions were present (Tables 6 and 7). Various levels of resistance/susceptibility of apple cultivars to apple scab have also been observed in two Swedish orchards located in Balsgård and Alnarp (Sandskär and Gustafsson 2004). In both orchards the cv. 'Priscilla' showed total resistance to the disease, however, single lesions were found on the cv. Novamac leaves in Balsgård. Our observations confirmed that the resistance of this cultivar had been lost, from the presence of heavily infected trees (orchard D, Table 4). Total resistance of unprotected cultivars with the gene *Rvi6* has been previously observed in Canadian inoculation tests (Quamme et al. 2005), which indicated a lack of 6 and 7 races in studied *V. inaequalis* populations. They also found that the absence of apple scab

Table 6 Variation in the populations of the fungus *Venturia inaequalis* in 2011 and 2012 based on the infection of indicator apple genotypes under greenhouse conditions

Differential hosts	2011				2012			
	(A)[a]	(B)	(D)	(E)	(A)	(B)	(D)	(E)
h(1)	++	++	++	++	++	++	++	++
h(2)				++				++
h(3)	+		+	+				++
h(4)								
h(5)								
h(6)	++	++	+		++	++	++	
h(7)								
h(8)			+	++	++		++	++
h(9)				+				+
h(10)		++	+	++			++	+++
h(11)								
h(12)								
h(13)				+				
h(14)								+
h(15)								

[a] Combined inoculum of *V. inaequalis* from the following locations: A, Pęczniew; B, Nowe Rowiska; D, Wola Branicka; and E, Dąbrowice (commercial orchard located close to orchard B)

+One or very few sporulating scab lesions detected on close inspection of the tree

++Several visible lesions which were present on various shoots

+++Heavy infection with numerous lesions on most leaves

Table 7 Variation in the populations of the fungus *Venturia inaequalis* in 2013 and 2014 based on the infection of indicator apple genotypes under the conditions of natural infections of apple trees (in an orchard)

Differential hosts	2013			2014		
	Miłobądz[a]	Brzezna	Dąbrowice	Miłobądz	Brzezna	Dąbrowice
h(1)	+++	+++	+++	++	++	++
h(2)	+++	+++	+++	++	++	
h(3)	+++	+++	+++	++	++	++
h(4)			++			++
h(5)						
h(6)	+	+				
h(7)						
h(8)	+++	+++	+++	++	++	++
h(9)	++		++	++		
h(10)	+++	+++	+++	++	++	++
h(11)						
h(12)						
h(13)			++			
h(14)			+			+
h(15)						

[a] Location of the commercial orchards: Miłobądz (near Gdańsk), Brzezna (near Nowy Sącz) and Dąbrowice (near Skierniewice)

+One or very few sporulating scab lesions detected on close inspection of the tree

++Several visible lesions which were present on various shoots

+++Heavy infection with numerous lesions on most leaves

symptoms on hosts containing the gene *Rvi5* was related to the absence of race 5. Our study showed various severity levels of apple scab infection on leaves of *Rvi6* cultivars in organic orchards, and no infection on such cultivars in orchards with IPM systems. This may be associated with the various efficacy of fungicides recommended for both systems. In general, copper and sulphur products are less effective against apple scab than synthetic products used in IPM systems (Holb et al. 2005). Holb (2007) confirmed that the same cultivar can show different reactions depending on the performance of the orchard system. They also showed that the disease severity was lower in IPM orchard systems compared to organic ones. The only exception was in case of scab resistant cultivars, which were well protected in both systems. Such absence of apple scab symptoms on *Rvi6* cultivars in IPM orchards, despite the presence of *V. inaequalis* pathotypes able to infect h(6), both in orchard and greenhouse tests (Tables 6 and 7), indicates the needs to protect the cultivars with *Rvi6* gene with a low number of fungicides. This would reduce the cost of apple production and limit the negative impact of chemical treatments on the environment (Holb et al. 2012).

It should be noted, however, that most of the apple cultivars grown are very susceptible to apple scab. Due to the limited number of chemical preparations permitted for use by organic orchards, the yield from such cultivars is often unsatisfactory. On the other hand, only about 3 % of *Rvi6* gene-containing cultivars is planted, in both IPM and organic orchards. However, in organics they predominate. Both our findings and those of other authors have shown that it is necessary to continue apple breeding aimed at producing scab resistant cultivars, particularly those with polygenic resistance which are also attractive to consumers.

Identification of virulent stains in populations of *V. inaequalis*

This study, conducted under greenhouse conditions on the 15 differential genotypes inoculated with mixed conidia of *V. inaequalis* originating from orchards

(Tables 1 and 2) located near Sieradz (Pęczniew) (A) and Zgierz (Wola Branicka) (D), and two orchards near Skierniewice (Nowe Rowiska and Dąbrowice) (B and E), suggested differences between the apple scab pathogen populations. The four inocula used in 2011 were able to infect the leaves of the host h(1) 'Golden Delicious', which carries the *Rvi1* (*Vg*) gene, at a medium or high level (Table 6). Earlier greenhouse studies in Canada, also based on mixed inocula, showed that both the number of lesions per leaf and the number of lesions per cm^2 on the leaf surface was sufficient to classify the 'Golden Delicious' cultivar as being moderately susceptible (Dewdney et al. 2003).

Sporulating lesions of apple scab were also found on the differential hosts h(3) (locations A, D and E), h(6) (A, B and D), h(8) (D and E), h(9) (E), h(10) (B, D and E) and h(13) (E). In addition, all the inocula used in 2012 were pathotypes capable of infecting differential genotypes carrying the *Rvi1* gene (Table 6). Furthermore, sporulating lesions of apple scab were detected on the differential hosts h(2) and h(3) (E), h(6) (A, B and D), h(8) (A, D and E), h(9) (E), h(10) (D and E) and h(14) (E). Inoculation with mixed inocula on the leaves of some hosts (e.g. h2 and h10) resulted in lesions resembling a hypersensitive response, or stellate necrosis and chlorosis with limited sporulation, in addition to the typical sporulating lesions. We suggest that this was related to the presence of various pathotypes of *V. inaequalis* in the mixed inocula used in this study.

The results showed that the level of leaf infection in case of inoculum from orchard B was similar to that observed for orchards A and D (Table 6). The severity of the scab both on susceptible hosts ('Gala') and on resistant hosts ('Priscilla' and 'A723–6') were comparable.

These results confirm that under controlled conditions, *Rvi6* virulent isolates were present in populations of the orchards A, B and D, and indicated that isolates virulent to other R-genes were also present.

A number of reports from around the world that evaluated single spore isolates have shown that there are differences in specific pathogenicity among isolates of *V. inaequalis*, which represent different physiological races of the pathogen (Bénaouf and Parisi 2000; Bus et al. 2005; Martínez-Bilbao and Murillo 2005; Sandskär and Liljeroth 2005; Williams and Brown 1968).

Identification of virulent isolates under orchard conditions

Scab infections that occurred under natural conditions in the orchards, located in Miłobądz near Tczew, Brzezna near Nowy Sącz and Dąbrowice near Skierniewice, showed differences among the populations of *V. inaequalis*. In 2013, sporulating scab lesions were found on the leaves of 10 out of the 15 differential genotypes. Pathotypes of the pathogen capable of infecting the differential hosts h(1), h(2), h(3), h(8) and h(10) were found in all of these three monitored populations, and caused heavy infections with numerous lesions on most leaves (Table 7). In addition, sporulating scab lesions were detected on h(6) (Miłobądz and Brzezna) and h(9) (Miłobądz and Dąbrowice), and there also were lesions present on h(4), h(13) and h(14) in the population of *V. inaequalis* originating from the orchard in Dąbrowice. On the leaves of h(4), h(9) and h(13) the lesions were visible and were concentrated on various shoots, while on the leaves of h(6) and h(14) only a single or few sporulating scab lesions were observed under close inspection of the tree. According to Soufflet-Freslon et al. (2008) h(14) also carries three quantitative trait loci (QTL) which are related to resistance. One of these is co-localised with the *Rvi14* gene on LG6, whereas the remaining two were detected on LG11 and LG17 and contributed to lower disease susceptibility. Putative pathotypes which are able to overcome the apple scab resistance of h(13) ('Durello di Forlì') have been reported earlier in Belgium (Lefrancq and Lateur 2009). However, an extensive study on *V. inaequalis* races has not been performed in Poland, but according to Lemaire et al. (2016), *Rvi6* strains from Poland are clustered either with genotypes from populations from Denmark/Sweden or France, while FLO (the wild *M. floribunda*, the wild progenitor of the *Rvi6* resistance) strains are clustered with the group of *Rvi6* strains sampled in France. In addition to this race, races (5), (6) and (7), which result in abundant sporulation, and races (2) and (4) which produce symptoms of a small number of infections only at high disease pressure, have been identified on specific indicator apple genotypes (Lefrancq and Lateur 2009). The occurrence of race (7) had also been detected earlier in Spain (Martínez-Bilbao and Murillo 2005), but has not yet been detected in Poland.

In 2014, a year with less favourable scab conditions than the 2013 season, scab was detected on four

differential hosts in all three locations, namely h(1), h(3), h(8) and h(10). In addition, sporulating scab spots appeared on the host h(2) in the orchards at Miłobądz and Brzezna (Table 7), on h(4) and h(14) at Dąbrowice and on h(9) at Miłobądz. The medium severity of apple scab was observed on the leaves of almost all the hosts, h(1), h(2), h(3), h(4), h(8), h(9) and h(10), in all locations. The lesions were immediately apparent and were generally concentrated in few parts of the tree. On h(14) only, a single or few sporulating scab lesions were detectable on close inspection of the tree. Research on differential genotypes conducted in the Czech Republic between 2006 and 2009 also showed the presence of apple scab symptoms on *Rvi6* cultivars in all four monitored locations (Vavra and Bocek 2010). Monitoring the occurrence of physiological races in *V. inaequalis* populations, based on potted trees of differential genotypes, has also contributed to race monitoring in Sweden, and has been confirmed to be a method for the portable screening of orchards (Sandskär and Liljeroth 2005). Those studies showed that six of the first seven races of the apple scab causal agent were found in Sweden, with the exception of race (5), which also remained undetected in our study.

In conclusion, the monitoring performed in this study allowed the first confirmation of races of *V. inaequalis* which were able to overcome the resistance conferred by the *Rvi6* gene in several apple orchards in Poland. Pathogenicity tests using differential genotypes allowed for the identification of isolates which were able to overcome some, but even all 10 of the 15 known resistance genes present in these differential hosts. The results indicate differences in the prevalence of virulent isolates among the different fruit-growing areas monitored in this study. There is a need to introduce basic chemical protection for scab-resistant cultivars of apple trees in order to prevent or delay the loss of resistance.

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